Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2016, Volume 4, Issue 12
ISSN: 2321–3647(online)

Determination of maturity stages in Labeo dyocheilus from Mid-Central Himalayan landscape, Uttarakhand

Rakesh Verma
Department of Zoology, Government Post Graduate College Pithoragarh, Uttarakhand, (India)

ABSTRACT

The maturity stages were determined in fish gonads generally by means of the macroscopic study (morphological description of developmental stages of the ovarian and testicular cycle) and microscopic study (histological description of developmental stages of the ovarian and testicular cycle). The different maturity stages determined by modified ICES scale and histological studies of gonads were done by a method described by Humason. In the present study the ovarian and testicular development of *Labeo dyocheilus* was observed and was divided into seven stages. Male and female fish mature at the same time in the year and show similar as well as same maturity stages. The name given to all stages were Stage I (Immature or Virgin), Stage II (Maturing virgin), Stage III (Developing), Stage IV (Maturing), Stage V (Ripening), Stage VI (Spawning or Running) and Stage VII (Spent).

Keywords: Central Himalaya, Labeo dyocheilus, maturity stages

*Corresponding Author Email: rakeshverma.pithoragarh@gmail.com
Received 08 November 2016, Accepted 21 November 2016

Please cite this article as: Verma R., Determination of maturity stages in Labeo dyocheilus from Mid-Central Himalayan landscape, Uttarakhand. American Journal of Pharmacy & Health Research 2016.

INTRODUCTION

The knowledge on the reproductive biology of fish is important for productive aquaculture and scientific based fishery management. It includes maturation biology, development of eggs, season and frequency of spawning, reproductive capacity and ecology of the spawning grounds. It is well known that the reproductive processes in fishes are controlled by endogenous biological rhythms as well as by environmental factors (Munro, 1990)[1]. In male fish, knowledge of spermatozoon ultra-structure has been used in systematic (Mattei, 1991)[2] and was found to be important in the assessment of milt quality (Billard, 1978)[3]. West (1992)[4] investigated that histological studies provide precise information on gonadal development. The importance of histological description of gametogenesis was emphasised by Booth and Weyl (2000)[5] who noted that histological staging must be validated if errors in the estimation of maturity and reproductive seasonality are to be minimised. There was a lot of literature of macroscopic and microscopic study of fish gonads as mentioned earlier. Smith and Walker (2004)[6] on common carp, Bisht et. al. (2005)[7] on *Schizothorax plagiostomus*, Kumar et. al. (2006)[8] on *Botia dayi*. etc. The present work deals with the macroscopic study (morphological description) and microscopic study (histological description) of ovarian and testicular cycle in *Labeo dyocheilus*.

MATERIALS AND METHOD

Sampling was done in the W. Ramganga River from where *Labeo dyocheilus* were collected (25-30 cm in length) for the period of two years. After taking morphometric measurements, the fish samples were preserved in 5 % formalin for further studies. The maturity stages were estimated on the basis of growth rate of ova throughout the year. The different maturity stages determined by ICES scale (Wood, 1930)[9]. Histological studies of gonads were done by a method described by Humason (1972)[10].

RESULTS AND DISCUSSION

In this study (Table 1 to 14), maturity stages were studed and classified on the basis of Wood's (1930)[9], the ovarian and testicular development of *Labeo dyocheilus* was divided into seven stages. The oogonia and spermatogonia pass through a number of maturation stages. Which involve complicated changes in cytology, cytoplasm and nucleus ratio in both gonads.

Table 1: Stage I of Ovary - Immature or Virgin

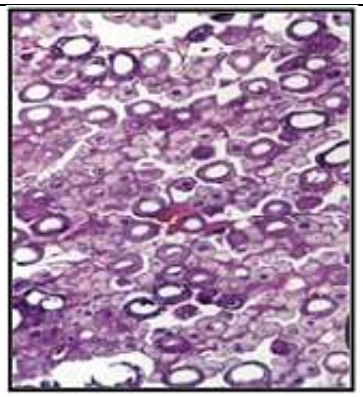
Macroscopic Study	Microscopic Study
	
Ova diameter from 3 to 25 Omd, which is a range, falls in the Wood's size group classification of immature 1st stage varying from 5-25 Omd. This size group is not visible by naked eyes. We obtained a peak at 20 Omd, which is an indicative of maximum number of ova of this diameter.	The germinal epithelium was with cytoplasm and deeply stained nucleus with several nucleoli. Oogonia lie in clusters and are attached to ovigirous folds. Each primary oogonium were smallest in size with centrally placed nucleus, deeply stained nucleoli and basophilic cytoplasm.

Table 2: Stage II of Ovary - Maturing virgin

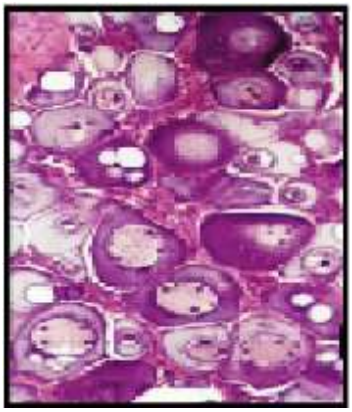
Macroscopic Study	Microscopic Study
	
The first batch of ova separated from the general egg stock with a diameter range of 5-55 Omd falls in the immature 2nd stage of Wood's classification. Under this range, maximum no. of ova with the diameter of 42 Omd was obtained.	Oogonia increases in size by cytoplasm absorption and considered as oocytes with nucleoli. Some sections showed extrusion of nucleoli from nucleus to cytoplasm. The stroma of the ovary was well developed, while the tunica albugenia was thick.

Table 3: Stage III of Ovary - Developing

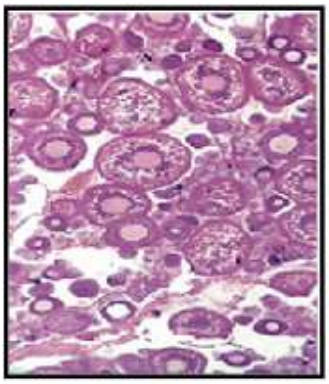
Macroscopic Study	Microscopic Study
	
Ovaries were slight dark yellow color and the ova collected were ranges from 15-80 Omd. This range is estimated as maturing 1st stage. A peak at 58 Omd was observed, which is an indicative of maximum number of ova of this diameter.	Appearance and randomly distribution of cortical alveoli in the cytoplasm. Further increases in size and number, shifting towards periphery forms layers. Primary oocytes were also seen, few have yolk vesicle. The nucleus-to-cytoplasm ratio increased.

Table 4: Stage IV of Ovary - Maturing

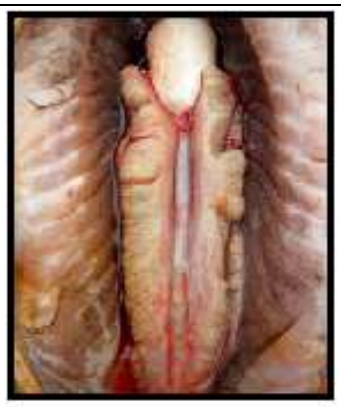
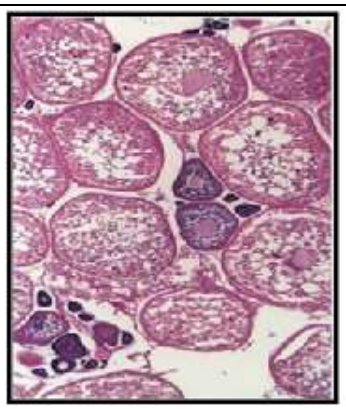
Macroscopic Study	Microscopic Study
	
The ova diameters lie in this category is varied from 25 to 100 Omd and thus this stage is estimated as maturing 2nd stage. Under this range, maximum number of ova with the diameter of 75 Omd was obtained.	Three types of oocytes were seen. Primary oocytes present between the crevices. Some secondary stage, mostly tertiary-stage oocytes, characterized by a well developed zona radiate with well developed true yolk granules and some fat droplets.

Table 5: Stage V of Ovary – Ripening

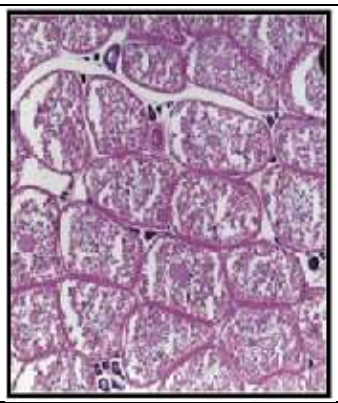
Macroscopic Study	Microscopic Study
	
The ovary samples fall in this stage slight dark yellow color and enlarge. The ova diameter varied from 35-140 Omd, which is a mature 1st stage. The maximum number of ova obtained in this range was the diameter of 110 Omd.	Yolk vesicles filling almost the entire ooplasm. The nucleus is reduced in size and nucleoli become smaller in size and fewer in number. The nuclear membrane becomes irregular. The primary oocytes and tertiary stage oocytes were seen. This is a clear-cut indication of the final maturity of the oocyte.

Table 6: Stage VI of Ovary - Spawning or Running

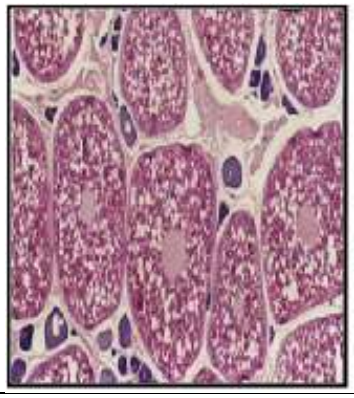
Macroscopic Study	Microscopic Study
	
Large sized ova with full yolk, come out by applying a slight pressure on the abdomen. The diameter of ova 60-170 Omd. Under this category, maximum no. of ova with the diameter of 155 Omd was obtained.	Histologically, the ovarian picture is more or less similar to that of stage 5 and fish has only two types of oocytes in this stage, which are primary and tertiary oocytes. Occasionally, a secondary oocyte was also seen.

Table 7: Stage VII of Ovary – Spent

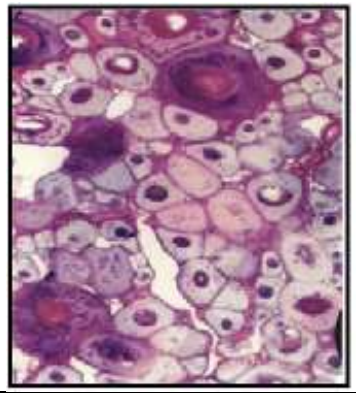
Macroscopic Study	Microscopic Study
	
Ovary got shrunken; flaccid, dirty light yellow in color and few large sized yellow ova were collected. The ova diameter fluctuated, ranging 15 to 60 Omd. The maximum no of ova were of the diameter 48 Omd.	The ovary show a large number of atretic oocytes in the resorption conditions with some mature eggs. The discharged follicles are also seen in the reabsorption condition. Empty spaces present in the ovary and between the ovigerous folds.

Table 8: Stage I of Testis - Immature or Virgin

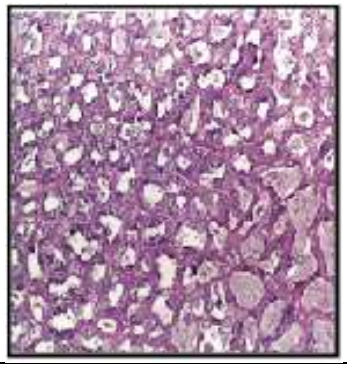
Macroscopic Study	Microscopic Study
	
The testes were thread like in structure, very light in colour, cylindrical in shape and extending about two third of the body cavity generally. In this condition, the testes not differentiated with naked eye.	Seminiferous lobuoles were enclosed in a thin peritoneum and has a fairly thick tunica. The stroma was made up of loose connective tissue, interstitial cells and blood capillaries showed periodic variations.

Table 9: Stage II of Testis - Maturing virgin

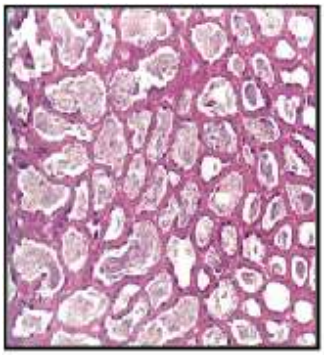
Macroscopic Study	Microscopic Study
	
At the stage II, the testis samples were slight thicker as compare to previous stage, slight whitish in colour and increased in volume due to cellualar mass increasement. Generally light colour testis look likes simple filament.	The tunica was thick, lobular structure was well established. No active spermatogenesis. Some lobules had a big and empty lobule lumen with no spermatogonia. Developed interstitial tissue contained myoid cells.

Table 10: Stage III of Testis – Developing

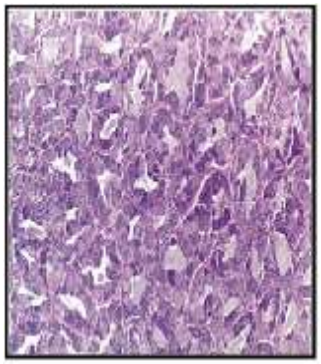
Macroscopic Study	Microscopic Study
	
The testes observed in this stage were thicker due to cellular increases, whitish in colour due to chemical changes and increased in volume and weight due to spermatogenesis process is about to actively start.	Lobules contained cysts and became elongated during this month. The tunica has become less thick than in the previous stage. Lobular demarcations were absolutely clear, and interstitial tissue was less marked.

Table 11: Stage IV of Testis – Maturing

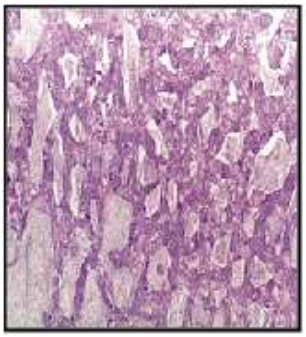
Macroscopic Study	Microscopic Study
	
At this stage, testes became much thicker due to cellular development, lobule were irregular in shape and irregular development, showing dark white in color throughout testis. Both testes dissimilar in shape.	The peripheral parts of the testis were less developed than the central part. Lobular boundaries were clearly demarcated and contained myoid cells. Interstitial tissue was well developed, sertoli cells were attached to the lobule boundary

Table 12: Stage V of Testis – Ripening

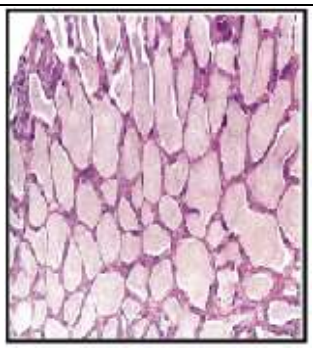
Macroscopic Study	Microscopic Study
	
The testes observed in this stage were deep white in color, having the maximum weight and occupied entire length of the body cavity	Lobules stored and filled with sperm, had the discontinuous type of germinal epithelium, showed that active spermatogenesis has ceased. Interstitial tissue and lobule boundaries was well developed contained myoid cells. The tunica was very thin.

Table 13: Stage VI of Testis - Spawning or Running

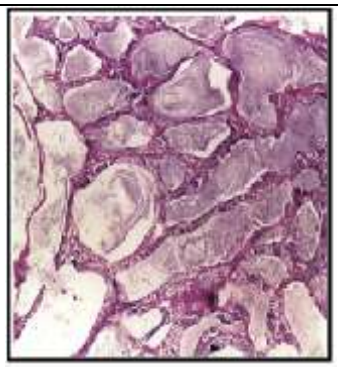
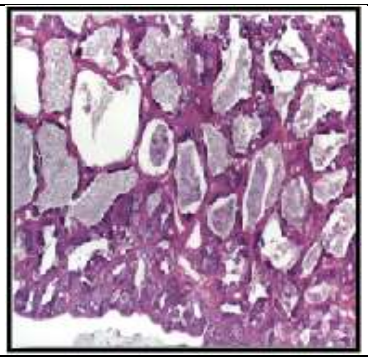
Macroscopic Study	Microscopic Study
	
The testis samples fall in this category were releasing milt by applying high pressure on abdomen. Milt was thick, white and adhesive	The lobules were full and extended with sperms. Secondary spermatocytes and spermatid were also present. Some lobules had only sperm in them. Peripheral lobules have cysts where a new wave of spermatogenesis was starting.

Table 14: Stage VII of Testis –Spent

Macroscopic Study	Microscopic Study
	
Morphologically the testes exhibit reduction in weight and volume during this period., the testes appeared flaccid and thin due to extruded milt and became thin, slenderical, translucent with poor vascularity	Few sperm were seen in the lumen of the lobules. Lumen was empty and tunica became slightly thicker, as compared to the earlier phase. The germinal epithelium was of the discontinuous type and the interstitium was well developed.

In the perspective of diversity in spawning behavior, Hickling and Rutenberg (1936)[11] studied the spawning behaviors based on the size distribution of intraovarian eggs in different fishes. For the determination of maturity stages, most of the ichthyologist follows the trend set in ICES scale proposed by Wood (1930)[9]. In the present work, seven maturity stages was determined for Labeo *dyocheilus*. Seven maturity stages are frequently observed in the literature for a number of fishes

viz. *Crossocheilus latius latius* by Negi and Dobriyal (1997)[12], *S. curvifrons* by Sunder (1984) [13] from Jhelum River, *P. sulcatus* by Thapliyal (2002)[14] from Alaknanda River, *Tor chelynoides* by Uniyal (2003)[15] from Western Nayar River, *N. botia* by Singh (2004)[16] from Khoh River, *Tor tor* by Desai (2000)[17] from Narmada River, *Tor tor* by Pathani (2000)[18] from Kumaun Himalaya, *Gudusia chapra* by Vinchi et. al, (2005)[19] etc. In present study gradual morphological changes in nucleolus and follicular epithelium was also observed in different stages of oocyte maturity. Nucleolus changes found by Thiry and Poncin (2005)[20] and follicular epithelium by Quagio-Grassiotto and Guimaraes (2003)[21] in developing oocyte. In present study development of the yolk in maturing oocyte has also noted. Similarly the development of the yolk also observed by Hartling and Kunkel (1999)[22] which confirm our observation. According to Schulz and Miura (2002)[23] teleost testis exhibited tremendous variations in structure, spermatogenic pattern and maturation during testicular cycle. In the present study, spermatogenesis was divided

CONCLUSION

Fish Labeo dyocheilus observed to show seven maturity stages in this study. Male and female fish show similar type of gonadal developmental stages round the study. Various morphological and histological changes had been observed during annual sexual cycle.

ACKNOWLEDGMENTS

The author acknowledges the Life Science Research Board, D.R.D.O. (R&D) New Delhi organization for financial assist and Department of Zoology, Government Post Graduate College Pithoragarh, Uttarakhand for laboratorial as well as technical support.

REFERENCES

1. Munro, A.D. Reproductive seasonality in teleosts: environmental influences. CRC Press, Boca Raton, Florida, USA, 1990: 1-11.
2. Mattei, X. Spermatozoa ultrastructure and its systematic implications in fishes, Canadian Journal of Zoology, 1991; 69: 3038-3055.
3. Billard, R. Changes in structure and fertilising ability of marine and freshwater fish spermatozoa diluted in media of various salinities," Aquaculture. 1978; 14: 187-198.
4. West, G. Methods of assessing ovarian development in fishes: a review, Australian Journal of Marine and Fresh water Research. 1992; 41: 199-222.
5. Booth, A.J. and Weyl, O.L.F. Histological validation of gonadal macroscopic staging criteria for Labeo cylindricus (Pisces: Cyprinidae)," African Zoology. 2000; 35: 223-231.

6. Smith, B.B. and K.F. Walker. Spawning dynamics of common carp in the River Murray South Australia, shown by macroscopic and histological staging of gonads. J. Fish Biol. 2004; 64: 336-354.
7. Bisht, K.L., Dobriyal, A.K., Joshi, H.K., Bahuguna, P.K. and Singh, H.R. Maturation biology and spawning ecology of Schizothorax plagiostomus (Pisces: Cyprinidae) from river Pinder, Uttaranchal. The Ekologia. 2005; 3: 89-97.
8. Kumar, K., Bisht, K.L., Dobriyal, A.K., Joshi, H.K. and Bahuguna, P.K. Maturation biology of a hillstream fish Botia dayi Hora from Garhwal Himalaya, Uttaranchal. Env. Cons. Journal. 2006; 7: 41-48.
9. Wood, H. Scottish herring shoals. Pre-spawning and spawning movements. Scotland Fish Bd. S. Invest. 1930; 1: 1-71.
10. Humanson, G. L. Animal Tissue Techniques, H. W. Freeman and Company, San Fransisco.1972; 0-7167-0692-X.
11. Hickling, C.F. & Rutenberg, E. The ovary as an indicator of spawning period in fishes. Mar. Biol. Assoc. 1936; 21: 311-412.
12. Negi, K.S. and Dobriyal, A.K. Sexual maturity and spawning ecology of a hillstream carp Crossocheilus latius latius (Ham) from the glacier fed river Mandakini, Garhwal, Central Himalaya. J. Inland Fish. Soc. India. 1997; 29: 26-33.
13. Sunder, S. Studies on the maturation and spawning of Schizothorax curvifrons Heckel from river Jhelum, Kashmir. J. Indian Inst. Sci. 1984; 65: 41-45.
14. Thapliyal, A. Some aspects of fish biology of Pseudecheneis sulcatus (McClelland) from Garhwal Himalaya, Uttaranchal. D.Phil. Thesis, HNB Garhwal University, Srinagar Garhwal. 2002.
15. Uniyal, S.P. Fish biology Investigations on Tor chelynoides (Mc Clelland) correlated with its habitat ecology in the Western Nayar. D. Phil. Thesis. HNB Garhwal University, Srinagar Garhwal. 2003.
16. Singh, R. Some aspects of fish biology and parasitology of Naemacheilus botia (Ham.) from Khoh River. D.Phil. Thesis HNB Garhwal University, Srinagar Garhwal. 2004.
17. Desai, V.R. Reproduction biology and spawning ecology of Tor tor (Hamilton) from the river Narmada. Ind Singh, H.R. and Lakra, W.S. (Edited): Coldwater aquaculture and Fisheries, New Delhi. 2000; 235-252.

18. Pathani, S.S. Reproduction biology of the golden mahseer, Tor putitora (Ham.) from Kumaun Himalayas. In. Singh, H.R. and Lakra, W.S. (Edited): Coldwater aquaculture and fisheries New Delhi. 2000; 253-264.
19. Vinchi, G.K., Suresh, V.R. and Bandyopadhyaya, M.K. Biology of Gudusia chapra (Ham.-buch.) from a floodplain wetland in West Bengal. Indian J. Fish. 2005; 52: 73-79.
20. Thiry, M. and Poncin, P. Morphological changes of the nucleolus during oogenesis in oviparous teleost fish, Barbus barbus. J. struct. Biol. 2005;152: 1-13.
21. Quagio-Grassiotto, I. and Guimaraes, A.C.D., , "Follicular epithelium, theca and egg envelope formation in Serrasalmus spilopleura (Teleostei, Characiformes, Characidae)," Acta Zool. 2003; 84: 121-129.
22. Hartling, R. C. and Kunkel, J.G. Developmental fate of the yolk protein lipovitellin in embryos and larvae of winter flounder, Pleuronectes americanus, J. exp. Zool. 1999; 284: 686-695.
23. Schulz, R.W. and Miura, T. Spermatogenesis and its endocrine regulation, Fish Physiol. Biochem. 2002; 26: 43-56.